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(54) Title: INHIBITORS OF β -LACTAMASES AND USES THEREFOR

(57) Abstract

The invention provides novel non- β -lactam inhibitors of β -lactamases. In particular, the invention provides such inhibitors which are boronic acids of formula (1) which is set forth in the specification. These compounds may be used with β -lactam antibiotics to treat β -lactam-antibiotic-resistant bacterial infections. Finally, the invention provides a pharmaceutical composition comprising these compounds.

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INHIBITORS OF β -LACTAMASES AND USES THEREFOR

BACKGROUND

5 Bacterial resistance to antibiotics has raised fears of an approaching medical catastrophe (Neu, *Science*, 257, 1064-1073 (1992)). Evolutionary selection and genetic transformation have made this problem pressing. Most antibiotic drugs are derivatives of naturally occurring bactericides (Davies, *Science*, 264, 375-382 (1994)), and many resistance mechanisms evolved long ago. Human use of antibiotics has refined these mechanisms and promoted their spread through gene transfer (Davies, *Science*, 264, 375-10 382 (1994)). A resistance mechanism originating in one species of bacteria can be expected to spread throughout the biosphere.

15 Bacterial adaptations to β -lactam drugs (e.g., amoxicillin, cephalothin, clavulanate, aztreonam) are among the best studied and most pernicious forms of antibiotic resistance. β -lactams target enzymes that are unique to bacteria and are thus highly selective. They have been widely prescribed. In the absence of resistance, β -lactams are the first choice for treatment in 45 of 78 common bacterial infections (*Goodman & Gilman's The Pharmacological Basis of Therapeutics* (Hardman et al., eds., McGraw-Hill, New York, 1996)). The evolution of resistance to these drugs has raised the cost of antibiotic therapy and reduced its effectiveness, leading to increased rates of morbidity and mortality.

20 β -lactam antibiotics inhibit bacterial cell wall biosynthesis (Tomasz, *Rev. Infect. Dis.*, 8, S270-S278 (1986)). The drugs form covalent complexes with a group of transpeptidases/carboxypeptidases called penicillin binding proteins (PBPs). PBP inactivation disrupts cell wall biosynthesis, leading to self-lysis and death of the bacteria.

25 Bacteria use several different mechanisms to escape from β -lactam drugs (Sanders, *Clinical Infectious Disease*, 14, 1089-1099 (1992); Li et al., *Antimicrob. Agents Chemother.*, 39, 1948-1953 (1995)). Probably the most widespread is the hydrolysis of β -lactams by β -lactamase enzymes.

30 TEM-1 and AmpC are two β -lactamases from *Escherichia coli*. *E. coli* is an important pathogen in its own right. It is the most common cause of gram-negative bacterial infection in humans (Levine, *New Engl. J. Med.*, 313, 445-447 (1985)), and is the most prevalent hospital-acquired infection (Thornberry, *Pharmacotherapy*, 15, S3-8 (1995)). *E. coli* that carry TEM-1, or for which AmpC production has been derepressed, are resistant to β -lactam treatment. As of 1992, as many of 30% of community-isolated

E. coli and 40-50% of hospital-acquired *E. coli* in the United States were resistant to β -lactams such as amoxicillin (Neu, *Science*, 257, 1064-1073 (1992)). Many of these resistant *E. coli* are resistant to β -lactamase inhibitors such as clavulanic acid and sulbactam.

5 TEM-1 and AmpC are major forms of plasmid-based and chromosomal β -lactamases and are responsible for resistance in a broad host range. The versions of TEM and AmpC (Galleni, et al., *Biochem. J.*, 250, 753-760 (1988)) in other bacterial species share high sequence identity to TEM-1 and AmpC from *E. coli*. TEM-1 structurally and catalytically resembles the class A β -lactamase from *Staphylococcus aureus*. The structures of AmpC from *Citrobacter freundii* and *Enterobacter cloacae* have been determined, and they closely resemble the structure of the *E. coli* enzyme (Usher, et al., *Biochemistry*, 37, 16082-16092 (1998)).

10 To overcome the action of β -lactamases, medicinal chemists have introduced compounds that inhibit these enzymes, such as clavulanic acid, or compounds that are less susceptible to enzyme hydrolysis, such as aztreonam. Both have been widely used in 15 antibiotic therapy (Rolinson, *Rev. Infect. Diseases* 13, S727-732 (1991)); both are β -lactams. Their similarity to the drugs that they are meant to protect or replace has allowed bacteria to evolve further, maintaining their resistance.

20 Resistance to these new classes of β -lactams has arisen through modifications of previously successful mechanisms. Point substitutions in β -lactamases allow the enzymes to hydrolyze compounds designed to evade them (Philippon et al., *Antimicrob. Agents Chemother.*, 33, 1131-1136 (1989)). Other substitutions reduce the affinity of β -lactam inhibitors for the enzymes (Saves, et al., *J. Biol. Chem.*, 270, 18240-18245 (1995)) or 25 allow the enzymes to simply hydrolyze them. Several gram positive bacteria, such as *Staph. aureus*, have acquired sensor proteins that detect β -lactams in the environment of the cell (Bennet and Chopra, *Antimicrob. Agents Chemotherapy*, 37, 153-158 (1993)). β -lactam binding to these sensors leads to transcriptional up-regulation of the β -lactamase. β -lactam inhibitors of β -lactamases, thus, can induce the production of the enzyme that they are meant to inhibit, defeating themselves.

30 It is noteworthy that the human therapeutic attack on bacteria has paralleled the path taken in nature. Several species of soil bacteria and fungi produce β -lactams,

presumably as weapons against other bacteria (although this remains a matter of debate).

Over evolutionary time, susceptible bacteria have responded to β -lactams with β -lactamases, among other defenses. In turn, soil bacteria have produced β -lactams that resist hydrolysis by β -lactamases or have produced β -lactams that inhibit the β -lactamases.

5 *Streptomyces clavuligeris* makes several β -lactams, including clavulanic acid, a clinically used inhibitor of class A β -lactamases such as TEM-1. *Chromobacterium violaceum* makes aztreonam, a clinically used monobactam that resists hydrolysis by many β -lactamases. One reason why bacteria have been able to respond rapidly with "new" 10 resistance mechanisms to β -lactams, and indeed many classes of antibiotics, is that the mechanisms are not in fact new. As long as medicinal chemistry focuses on new β -lactam molecules to overcome β -lactamases, resistance can be expected to follow shortly. The logic will hold for any family of antibiotic where the lead drug, and resistance mechanisms to it, originated in the biosphere long before their human therapeutic use. This includes the aminoglycosides, chloramphenicol, the tetracyclines and vancomycin.

15 One way to avoid recapitulating this ancient "arms race" would be to develop inhibitors that have novel chemistries, dissimilar to β -lactams. These non- β -lactam inhibitors would not themselves be degraded by β -lactamases, and mutations in the enzymes should not render them labile to hydrolysis. Novel inhibitors would escape detection by β -lactam sensor proteins that up-regulate β -lactamase transcription, and may 20 be unaffected by porin mutations that limit the access of β -lactams to PBPs. Such inhibitors would allow current β -lactam drugs to work against bacteria where β -lactamases provide the dominant resistance mechanism.

It has previously been reported that boric acid and certain phenyl boronic acids are 25 inhibitors of certain β -lactamases. See, Kiener and Waley, *Biochem. J.*, **169**, 197-204 (1978) (boric acid, phenylboronic acid (2FDB) and *m*-aminophenylboronate (MAPB)); Beesley et al., *Biochem. J.*, **209**, 229-233 (1983) (twelve substituted phenylboronic acids, including 2-formylphenylboronate (2FORMB), 4-formylphenylboronate (4FORMB), and 4-methylphenylboronate (4MEPB)); Amicosante et al., *J. Chemotherapy*, **1**, 394-398 (1989) (boric acid, 2FDB, MAPB and tetraphenylboronic acid). More recently, *m*-30 (dansylamidophenyl)-boronic acid (NSULFB) has been reported to be a submicromolar inhibitor of the *Enterobacter cloacae* P99 β -lactamase. Dryjanski and Pratt,

Biochemistry, 34, 3561-3568 (1995). In addition, Strynadka and colleagues used the crystallographic structure of a mutant TEM-1 enzyme-penicillin G complex to design a novel alkylboronic acid inhibitor [(1R)-1-acetamido-2-(3-carboxyphenyl)ethane boronic acid] with high affinity for this enzyme. Strynadka et al., *Nat. Struct. Biol.*, 3, 688-695 (1996).

5

Finally, Weston et al. describe the testing of 37 boronic acids for inhibition of *E. coli* AmpC β -lactamase. Weston et al., *J. Med. Chem.*, 41, 4577-4586 (1998). The activity of the compounds varied considerably, with benzo-*b*-thiophene-2-boronic acid (BZBTH2B) being the most potent inhibitor ($K_i = 27$ nM). Using the previously-determined structure of the AmpC-MAPB complex (see Usher et al., *Biochemistry*, 37, 16082-16092 (1998)), several of the inhibitors were modeled into the AmpC binding site, and certain aspects of the interactions of the inhibitors with the enzyme were identified. The article concluded, however, that the modeling carried with it some ambiguities and that key questions regarding the structural bases for activity remained unanswered.

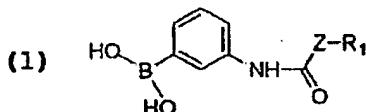
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SUMMARY OF THE INVENTION

The invention provides non- β -lactam inhibitors of β -lactamases. In particular, the invention provides β -lactamase inhibitors having the formula:

20



wherein:

25

R_1 is lower alkyl, lower alkyl substituted with one or more halogen atoms, a cyclic alkene, or a heterocyclic alkene, wherein the cyclic alkene or heterocyclic alkene may be substituted with one or more substituents R_2 ;

30

each R_2 is independently H, a halogen atom, lower alkyl, lower alkyl substituted with one or more halogen atoms, NH, NO, NO₂, CN, N-lower alkyl, N-lower alkyl substituted with one or more halogen atoms, OH, O-lower alkyl, O-lower alkyl substituted with one or more halogen atoms, CO-lower alkyl, CO-lower alkyl substituted with one

or more halogen atoms, COOH, lower alkyl-COOH, CONH₂, CON-lower alkyl, SO₃H, SO₂NH₂, or SO₂N-lower alkyl; and

Z is a bond, O, S, lower alkyl radical, or lower heteroalkyl radical.

The invention also provides a method of treating a β -lactam-antibiotic-resistant bacterial infection. The method comprises administering to an animal suffering from such an infection an effective amount of a β -lactamase inhibitor of formula (1), or a pharmaceutically-acceptable salt thereof, and an effective amount of a β -lactam antibiotic.

Finally, the invention provides pharmaceutical compositions comprising compounds of formula (1), or pharmaceutically-acceptable salts thereof, and a pharmaceutically-acceptable carrier. The pharmaceutical compositions may also comprise β -lactam antibiotics.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-B. Diagrams of the synthesis of compounds of formula (1).

DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EMBODIMENTS OF THE INVENTION

As used herein, the following terms have the following meanings.

"Lower alkyl" means a straight-chain or branched-chain alkyl containing 1-4 carbon atoms.

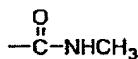
"N-lower alkyl" means N with one or more lower alkyls attached, such as -NHCH₃ or -N(CH₃)₂.

"O-lower alkyl" means O with a lower alkyl attached, such as -OCH₃.

"CO-lower alkyl" means C=O with a lower alkyl attached to the C, such as -COCH₃.

"Lower alkyl-COOH" means -COOH preceded by a lower alkyl radical, such as -CH₂COOH.

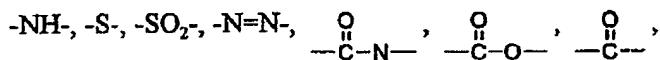
"CON-lower alkyl" means CON having a lower alkyl attached to the N, such as



"SO₂N-lower alkyl" means SO₂N having a lower alkyl attached to the N, such as -SO₂NHCH₃.

"Lower heteroalkyl" means a lower alkyl, as defined above, containing one or more atoms of S, N or O in the chain.

"Cyclic alkene" means a structure containing 1 or 2 rings, each ring containing 5 or 6 carbon atoms and at least one double bond. One or both of the rings may be aromatic. If the cyclic alkene contains more than one ring, the rings may be fused, connected by a bond, or connected by a linker, L. Preferably, L is a short chain containing up to six atoms in the chain. Suitable linkers include -O-,



lower alkyl radical (e.g., -CH₂-), lower alkene (e.g.,

-CH=CH-), lower alkyne (e.g., -C≡C-), and combinations thereof.

"Heterocyclic alkene" means a cyclic alkene, as defined above, wherein one or both of the rings contain(s) one or more S, N or O atoms.

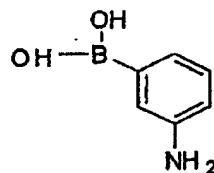
"Lower alkene" means a straight-chain or branched-chain alkene containing 2-4 carbon atoms.

"Lower alkyne" means a straight-chain or branched-chain alkyne containing 2-4 carbon atoms.

Compounds of formula (1) according to the invention include all optical isomers.

The compounds of formula (1) can be synthesized as described below. Unless otherwise noted, the various chemicals used in the syntheses described below are available from commercial sources including Aldrich Chemical, Milwaukee, WI, Lancaster Synthesis, Windham, NH, TCI America, Portland, OR, Sigma Chemical Co., St. Louis, MO, Acros Organics, Pittsburgh, PA, Chemservice Inc., West Chester, PA, BDH Inc., Toronto, Canada, Fluka Chemical Corp., Ronkonkoma, NY, Pfaltz & Bauer, Inc., Waterbury, CT, Avocado Research, Lancashire, UK, Crescent Chemical Co., Hauppauge, NY, Fisher Scientific Co., Pittsburgh, PA, Fisons Chemicals, Leicestershire, UK, ICN Biomedicals, Inc., Costa Mesa, CA, Pierce Chemical Co., Rockford, IL, Riedel de Haen AG, Hannover, Germany, Wako Chemicals USA, Inc., Richmond, VA, Maybridge Chemical Co. Ltd., Cornwall, UK, Bionet, Cornwall, UK, Trans World Chemicals, Inc., Rockville, MD, Apin Chemicals Ltd., Milton Park, UK, and Parish Chemical Co., Orem, UT.

Compounds of formula (1) can be synthesized using



and $R_1\text{-COCl}$ as starting materials. Examples of these syntheses are depicted in Figures

5 1A-B.

The synthesis depicted in Figure 1A is performed as follows. The polystyrene resin, P, functionalized with a 3-benzyloxy-1,2-propanediol (5 mmol; 1 mmol/g; 5g) as described in Leznoff and Wong, *Can. J. Chem.*, 51:3756-3764 (1973), is swollen in anhydrous acetone. Alternatively, functionalized resins can be purchased from Novabiochem. Then, 3-aminophenylboronic acid (10 mmol; 0.93g) is dissolved in anhydrous acetone and then added to the resin suspension, and the suspension is agitated. Anhydrous sodium sulfate can be used to absorb liberated water. Then, the resin is filtered and washed several times with water (to remove sodium sulfate and ethanol) and then with ether. The resin is divided into batches (20 mg of resin each). The resin is suspended in N-methyl pyrrolidone (NMP) and then the carbonyl chloride (10 mmol), previously dissolved in NMP, is added to the suspension. Diisopropylamine (DIEA; 20 mmol) is added. The reactions are mixed for 3-4 hours. Then, excess reagents are washed away. The final products are cleaved from the resin using acidic conditions and the resin is then separated by filtration. The solution is evaporated or extracted to give the final product.

The synthesis depicted in Figure 1B is performed as follows. First, 3-aminophenylboronic acid hemisulfate (20 mg; 0.11 mmol) is dissolved in CHCl_3 (6 ml). Poly(vinylpyridine) (0.33 mmol; 8.8 mmol/g; 0.037g) is added to the solution. $R_1\text{COCl}$ (0.165 mmol) is added, and the reaction agitated using a platform shaker until the presence of starting material is no longer detected by thin layer chromatography (TLC) (3-4 hours). Then, aminomethylated-polystyrene (0.28 mmol; 1 mmol/g; 0.28g) is added to scavenge the excess carbonyl chloride. The reaction is agitated for another 3-4 hours. The reaction is then filtered and concentrated to give the final product.

The compounds of formula (1) may contain an acidic or basic functional group and are, thus, capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable acids and bases. The term "pharmaceutically-acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic acid and base addition salts of compounds of formula (1). These salts can be prepared by reacting the purified compound with a suitable acid or base. Suitable bases include the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation, ammonia, or a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like. Representative acid addition salts include the hydrobromide, hydrochloride, sulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthalate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like.

The compounds of formula (1), and the pharmaceutically-acceptable salts thereof, are inhibitors of β -lactamases. The compounds of formula (1) may also prevent transcriptional up-regulation of β -lactamases and may be antibacterial by themselves, since it is likely that they will bind to PBPs which resemble β -lactamases.

Assays for the inhibition of β -lactamase activity are well known in the art. For instance, the ability of a compound to inhibit β -lactamase activity in a standard enzyme inhibition assay may be used (see, e.g., Example 2 below and M.G. Page, *Biochem J.* 295 (Pt. 1) 295-304 (1993)). β -lactamases for use in such assays may be purified from bacterial sources or, preferably, are produced by recombinant DNA techniques, since genes and cDNA clones coding for many β -lactamases are known. See, e.g., S.J. Cartwright and S.G. Waley, *Biochem J.* 221, 505-512 (1984). Alternatively, the sensitivity of bacteria known, or engineered, to produce a β -lactamase to an inhibitor may be determined. Other bacterial inhibition assays include agar disk diffusion and agar dilution. See, e.g., W.H. Traub & B. Leonhard, *Chemotherapy* 43, 159-167 (1997). Inhibition includes both reduction and elimination of β -lactamase activity.

5 The compounds of formula (1) may also be effective against bacteria resistant to β -lactam antibiotics as a result of porin mutations. Porin mutations are mutations in the proteins which form porin channels in bacterial cell walls. These mutations reduce the ability of β -lactam antibiotics to enter bacterial cells in which the mutations occur, thereby
10 making the bacteria resistant to these antibiotics. The presence of a porin mutation can be detected by polymerase chain reaction analysis of porin genes, polyacrylamide gel electrophoresis of a preparation obtained by mild osmotic shock (e.g., treatment with hypotonic solution containing EDTA, followed by gentle centrifugation and separation of the supernatant) of the bacteria (absence of a protein of the appropriate molecular weight being indicative of a porin mutation), or by determining resistance to infection by bacteriophage T₁A (a standard test for OmpF porin mutations).

15 The compounds of formula (1), or pharmaceutically-acceptable salts thereof, can be used to treat β -lactam-antibiotic-resistant bacterial infections. " β -lactam-antibiotic-resistant bacterial infection" is used herein to refer to an infection caused by bacteria resistant to treatment with β -lactam antibiotics due primarily to the action of a β -lactamase. Resistance to β -lactam antibiotics can be determined by standard antibiotic sensitivity testing. The presence of β -lactamase activity can be determined as is well known in the art (see above). Alternatively, and preferably, the sensitivity of a particular bacterium to the combination of a compound of formula (1), or a pharmaceutically-
20 acceptable salt thereof, and a β -lactam antibiotic can be determined by standard antibiotic sensitivity testing methods.

25 To treat a β -lactam resistant bacterial infection, an animal suffering from such an infection is given an effective amount of a compound of formula (1), or a pharmaceutically-acceptable salt thereof, and an effective amount of a β -lactam antibiotic. The compound of formula (1), or a pharmaceutically-acceptable salt thereof, and the antibiotic may be given separately or together. When administered together, they may be contained in separate pharmaceutical compositions or may be in the same pharmaceutical composition.

30 Many suitable β -lactam antibiotics are known. These include cephalosporins (e.g., cephalothin), penicillins (e.g., amoxicillin), monobactams (e.g., aztreonam), carbapenems (e.g., imipenem), carbacephems (loracarbef), and others. β -lactam antibiotics are effective

(in the absence of resistance) against a wide range of bacterial infections. These include those caused by both gram-positive and gram-negative bacteria, for example, bacteria of the genus *Staphylococcus* (such as *Staphylococcus aureus* and *Staphylococcus epidermidis*), *Streptococcus* (such as *Streptococcus agalactiae*, *Streptococcus pneumoniae* and *Streptococcus faecalis*), *Micrococcus* (such as *Micrococcus luteus*), *Bacillus* (such as *Bacillus subtilis*), *Listerella* (such as *Listerella monocytogenes*), *Escherichia* (such as *Escherichia coli*), *Klebsiella* (such as *Klebsiella pneumoniae*), *Proteus* (such as *Proteus mirabilis* and *Proteus vulgaris*), *Salmonella* (such as *Salmonella typhosa*), *Shigella* (such as *Shigella sonnei*), *Enterobacter* (such as *Enterobacter aerogenes* and *Enterobacter facium*), *Serratia* (such as *Serratia marcescens*), *Pseudomonas* (such as *Pseudomonas aeruginosa*), *Acinetobacter* such as *Acinetobacter anitratus*), *Nocardia* (such as *Nocardia autotrophica*), and *Mycobacterium* (such as *Mycobacterium fortuitum*). Effective doses and modes of administration of β -lactam antibiotics are known in the art or may be determined empirically as described below for the compounds of formula (1).

To treat an animal suffering from a β -lactam-antibiotic-resistant bacterial infection, an effective amount of a compound of formula (1), or a pharmaceutically-acceptable salt thereof, is administered to the animal, in combination with a β -lactam antibiotic. Effective dosage forms, modes of administration and dosage amounts of a compound of formula (1), may be determined empirically, and making such determinations is within the skill of the art. It is understood by those skilled in the art that the dosage amount will vary with the activity of the particular compound employed, the severity of the bacterial infection, the route of administration, the rate of excretion of the compound, the duration of the treatment, the identity of any other drugs being administered to the animal, the age, size and species of the animal, and like factors well known in the medical and veterinary arts. In general, a suitable daily dose will be that amount which is the lowest dose effective to produce a therapeutic effect. The total daily dosage will be determined by an attending physician or veterinarian within the scope of sound medical judgment. If desired, the effective daily dose of a compound of formula (1), or a pharmaceutically-acceptable salt thereof, may be administered as two, three, four, five, six or more sub-doses, administered separately at appropriate intervals throughout the day. Treatment of a β -lactam-

antibiotic-resistant bacterial infections according to the invention includes mitigation, as well as elimination, of the infection.

Animals treatable according to the invention include mammals. Mammals treatable according to the invention include dogs, cats, other domestic animals, and humans.

5 Compounds of formula (1) or pharmaceutically-acceptable salts thereof, may be administered to an animal patient for therapy by any suitable route of administration, including orally, nasally, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually. The preferred routes of administration are orally and parenterally.

10 While it is possible for the active ingredient(s) (one or more compounds of formula (1), or pharmaceutically-acceptable salts thereof, alone or in combination with a β -lactam antibiotic) to be administered alone, it is preferable to administer the active ingredient(s) as a pharmaceutical formulation (composition). The pharmaceutical compositions of the invention comprise the active ingredient(s) in admixture with one or more pharmaceutically-acceptable carriers and, optionally, with one or more other compounds, drugs or other materials. Each carrier must be "acceptable" in the sense of being 15 compatible with the other ingredients of the formulation and not injurious to the patient.

20 Pharmaceutical formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. Regardless of the route of administration selected, the active ingredient(s) are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

25 The amount of the active ingredient(s) which will be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration and all of the other factors described above. The amount of the active ingredient(s) which will be combined with a carrier material to produce a single dosage form will generally be that amount of the active ingredient(s) which is the lowest dose effective to produce a therapeutic effect.

30 Methods of preparing pharmaceutical formulations or compositions include the step of bringing the active ingredient(s) into association with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly

and intimately bringing the active ingredient(s) into association with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of the active ingredient(s). The active ingredient(s) may also be administered as a bolus, 10 electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient(s) is/are mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; 15 (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as 20 quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case 25 of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, 30 gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl

cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredient(s) moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient(s) therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient(s) can also be in microencapsulated form.

Liquid dosage forms for oral administration of the active ingredient(s) include pharmaceutically-acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient(s), the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active ingredient(s), may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan

esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by 5 mixing the active ingredient(s) with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or salicylate and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active ingredient(s). Formulations of the present invention which are suitable for vaginal administration also 10 include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of the active 15 ingredient(s) include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active ingredient(s) may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to the active 20 ingredient(s), excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to the active ingredient(s), excipients 25 such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery 30 of the active ingredient(s) to the body. Such dosage forms can be made by dissolving, dispersing or otherwise incorporating the active ingredient(s) in a proper medium, such as an elastomeric matrix material. Absorption enhancers can also be used to increase the flux of the active ingredient(s) across the skin. The rate of such flux can be controlled by

either providing a rate-controlling membrane or dispersing the active ingredient(s) in a polymer matrix or gel.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise the active ingredient(s) in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as wetting agents, emulsifying agents and dispersing agents. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like in the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of the active ingredient(s), it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the active ingredient(s) then depends upon its/their rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of parenterally-administered active ingredient(s) is accomplished by dissolving or suspending the active ingredient(s) in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the active ingredient(s) in biodegradable polymers such as polylactide-polyglycolide. Depending on

the ratio of the active ingredient(s) to polymer, and the nature of the particular polymer employed, the rate of release of the active ingredient(s) can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the active ingredient(s) in 5 liposomes or microemulsions which are compatible with body tissue. The injectable materials can be sterilized for example, by filtration through a bacterial-retaining filter.

The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a lyophilized condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately 10 prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the type described above.

The pharmaceutical compositions of the present invention may also be used in the form of veterinary formulations, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), 15 tablets, boluses, powders, granules or pellets for admixture with feed stuffs, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension or, when appropriate, by intramammary injection where a suspension or solution is introduced into the udder of the animal via its teat; (3) topical application, for example, as a cream, 20 ointment or spray applied to the skin; or (4) intravaginally, for example, as a pessary, cream or foam.

EXAMPLES

EXAMPLE 1: Synthesis of Compounds

25 The compounds listed in Table 1B below were synthesized as follows. See also the discussion above and Figure 1B.

The synthesis was carried out in liquid phase in parallel on a 10-position platform shaker. For the synthesis of a single compound, 0.030 g (0.161 mmol) of 3-aminophenylboronic acid hemisulfate (Aldrich) was dissolved in anhydrous chloroform 30 (CHCl₃; 6 mL), and the solution was stirred at room temperature. Then, 0.054 g poly-(4-vinylpyridine) resin (0.483 mmol; 8.8 mmol/g; crosslinked with 2% divinylbenzene; 60

mesh; purchased from Fluka) was slowly added to the stirring solution. Different acyl chlorides (R_1COCl ; 1.5 eq., 0.241 mmol; purchased from Aldrich, Maybridge International, TCI-US, Lancaster and Fluka) were then added, and the mixtures were stirred until TLC no longer detected the presence of starting material (from 12 to 24 hours). Then, aminomethylated polystyrene resin (3 eq., 0.483 mmol; 1.33 mmol/g; 0.363 g; purchased from Novabiochem) was added to the reaction solutions to scavenge the excess acyl chloride, and stirring was continued for 4-5 hours. Finally, the resins were filtered off, and the filtrate concentrated under vacuum to give the final compounds.

Compounds 2, 9, 10, 11 and 12 were separated from starting materials as follows. The solution was acidified with 4N HCl and then extracted with dichloromethane (CH_2Cl_2) three times to remove the unreacted 3-aminophenylboronic acid. The combined organic layers were dried over sodium sulfate (Na_2SO_4), and the solvent was removed under vacuum. The collected product was then triturated and washed with ethyl ether.

The purity of all synthesized compounds was determined by TLC using silica gel 60 F_{254} plates (Merck) with the appropriate solvent system. The chromatograms were visualized with a UV/visible lamp at λ 254 nm and 366 nm. Structures of the most active compounds were characterized by NMR spectroscopy and/or mass spectrometry. The purity and yield of the compounds listed in Table 1B are given in the following table.

Compound	Purity %	Chloride eq.*	Yield%
1	60	1.5	13
2	70	1.5	6
3	80	1.5	47
4	70	1.5	9
5	70	3	18
6	80	3	21
7	70	1.5	70
8	70	1.5	7
9	80	1.5	38
10	70	1.5	13
11	90	1.5	28
12	80	1.5	18
13	70	1.5	42
14	70	1.5	10

**EXAMPLE 2: Testing Of Compounds For
Inhibition Of β -Lactamases**

5 Compounds were tested for inhibition of TEM-1 and AmpC β -lactamases from *E. coli* using a spectrophotometric assay (Page, *Biochem. J.*, **295**, 295-304 (1993))

10 AmpC was expressed in *E. coli* JM109 cells (ATCC 53323) in which the native AmpC gene was attenuated or completely removed (obtained from Larry Blaszcak, Eli Lilly and Co., Indianapolis, Indiana) as described in Usher et al., *Biochemistry* **37**, 16082-16092 (1998). Briefly, DNA coding for the enzyme was located on a plasmid under the control of a temperature sensitive repressor. Cells containing this plasmid were grown in 2 liters of LB broth in a fermentor to log phase. Enzyme expression was then induced by temperature shock, and the cells were allowed to grow overnight. AmpC protein was purified from the supernatant over an Affigel-10 aminophenyl boronate affinity column 15 (Bio-Rad Laboratories, 1000 Alfred Nobel Drive, Hercules, CA). The purity of the sample was estimated by HPLC to be 96% or better. The amount of enzyme produced was estimated to be 150 mg based on absorbance at 280 nm.

20 TEM-1 was provided by Natalie Strynadka, University Of Alberta, Edmonton, Canada. Alternatively, TEM-1 may be produced as follows. The TEM-1 gene is cloned into HpaI site of pALTER-EX2 (Promega). The gene is under control of the T7 promoter which is turned on for protein expression. TEM-1 may be expressed in JM109 cells, as well as several other *E. coli* strains. Cells are grown to late log phase, followed by induction of protein expression. The cells are spun down and the supernatant, into which the enzyme has been exported, is collected. Because the enzyme has been exported into 25 the supernatant, purification may be achieved using standard column chromatography, as described in Matagne et al. *Biochem J.* **265**, 131-146 (1990); Escobar et al, *Biochemistry* **33**, 7619-7626 (1994).

30 Initial stock solutions of 1-100 mM concentrations of each compound to be tested were prepared in DMSO (dimethyl sulfoxide). Solubility and absorbance profiles were determined by incremental addition of small volumes of DMSO stock solutions to assay buffer (50 mM phosphate, pH 7.0) at 25° C using an HP8543 UV/Visible spectrophotometer with multi-cell transport running HP ChemStation software (version

2.5). Enzymatic testing was typically started at an upper concentration limit determined by the solubility and absorbance profile of the compound.

Standard assay conditions for AmpC were as follows: pH 7.0; 100 μ M cephalothin, sodium salt, as substrate; reaction monitored at 265 nm (cephalothin β -lactam absorbance peak); T = 25 °C; 50 mM phosphate buffer; no incubation of inhibitor with enzyme; cycle times of 10-15 seconds; total reaction volume = 1 mL; run time = 5 minutes; reaction initialized with addition of 0.06 nM AmpC. The background rate of cephalothin hydrolysis under these conditions was found to be two to three orders of magnitude less than the rate of the enzyme-mediated cephalothin hydrolysis, so no correction for background hydrolysis of substrate was used. For TEM-1, 100 μ M 6- β -furylacryloylamidopenicillanic acid, triethylammonium salt (FAP), was used as the substrate, the reaction was monitored at 340 nm (FAP β -lactam absorbance peak) and the cycle time was increased to 25 seconds (since this substrate was somewhat light sensitive). Due to the light sensitivity of FAP, the background rate of hydrolysis for this substrate was found to be minimal, but not insignificant, so all measured control and inhibited cell rates were corrected by subtraction of the FAP background rate. All other conditions for the TEM-1 assays were identical to those for the AmpC assays. DMSO was added to enzyme controls in all cases. Standard 1 mm path length quartz spectrophotometric cells (Hellma Cells, Inc., Jamaica, NY) were used in the assays. All assays were performed on the same HP8543 spectrophotometer noted earlier.

Linear and quadratic fits to the absorbance data for the full time course of each reaction were used to determine the reaction rate for each spectrophotometric cell. The resulting reaction rate data were used to calculate the inhibition constants for each potential inhibitor using the method of Waley (S.G. Waley, *Biochem. J.* 205, 631-633 (1982)). Briefly, this method involves the use of the integrated Michaelis-Menten equation to calculate K_i values for enzyme inhibitors from a comparison of the reaction rates of uninhibited and inhibited enzymatic reactions.

Specificity testing was performed by assaying the activity of an inhibitor against α -chymotrypsin (bovine pancreatic), β -trypsin (bovine pancreatic), and elastase (porcine pancreatic). Substrates for α -chymotrypsin (N-benzoyl-L-tyrosine ethyl ester, BTEE) and β -trypsin (N-benzoyl-L-arginine ethyl ester, BAEE) were purchased from Sigma

Chemical, St. Louis, MO. The elastase substrate used (elastase substrate 1, Na-methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide, was purchased from Calbiochem, San Diego, CA. All enzymes used for specificity testing were purchased from Sigma Chemical, St. Louis, MO. For α -chymotrypsin, 3 μ l of a 1 mg/ml enzyme stock solution (50 mM phosphate buffer, pH 7) was incubated with the boronate being tested for 5 minutes; then the reaction was initialized by addition of 630 μ M BTEE from a DMSO stock solution. The reaction was performed at 25°C and monitored at 260 nm. For β -trypsin, 40 μ l of a 0.8 mg/ml enzyme stock solution (50 mM phosphate buffer, pH 7) was incubated with the boronate being tested for 5 minutes; then the reaction was initialized by addition of 600 μ M BAEE from a DMSO stock solution. For elastase, 50 μ l of a 1 mg/ml enzyme stock solution (50 mM phosphate buffer, pH 7) was incubated with the boronate being tested for 5 minutes; then the reaction was initialized by addition of 64 μ M elastase substrate 1 from a DMSO stock solution.

The results of the testing are presented in Tables 1A and 1B below. Certain prior art compounds (Table 1A) were tested for comparative purposes. All of these compounds were obtained from Lancaster Synthesis, Windham, NH, except for MAPB which was obtained from Sigma, St. Louis, MO. All of these compounds were used as is with no additional purification or verification performed. The compounds in Table 1B were synthesized as described in Example 1.

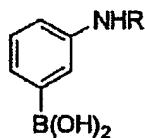
20

TABLE 1A

boronate	Ki <i>E. coli</i> AmpC (μ M)	Ki <i>E. coli</i> TEM-1 (μ M)
NSULFB	1.6	88.0
4FORMB	2.8	35.0
4MEPB	5.2	>100
MAPB	5.8	>>100
2FDB	8.0	>100
2FORMB	62.0	>100

NSULFB = *m*-(dansylamidophenyl)-boronic acid; 4FORMB = 4-formylphenylboronate; 4MEPB = 4-methylphenylboronate; MAPB = *m*-aminophenylboronate; 2FDB = phenylboronic acid; and 2FORMB = 2-formylphenylboronate.

TABLE 1B



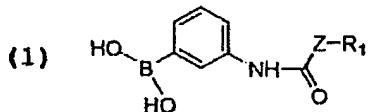
<u>Number</u>	<u>R</u>	<u>Ki AmpC (μM)</u>
1		1.6
2		2.5
3		4.0
4		4.0
5		7.2
6		8.2
7		8.2

<u>Number</u>	<u>R</u>	<u>Ki AmpC (μM)</u>
8		8.3
9		8.3
10		12.3
11		12.0
12		14.1
13		16.5
14		25

WE CLAIM:

1. A method of treating a β -lactam-antibiotic-resistant bacterial infection comprising administering to an animal suffering from such an infection an effective amount of a compound having the formula:

5



wherein:

10 R_1 is lower alkyl, lower alkyl substituted with one or more halogen atoms, a cyclic alkene, or a heterocyclic alkene, wherein the cyclic alkene or heterocyclic alkene may be substituted with one or more substituents R_2 ;

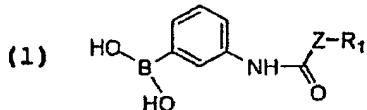
15 each R_2 is independently H, a halogen atom, lower alkyl, lower alkyl substituted with one or more halogen atoms, NH, NO, NO₂, CN, N-lower alkyl, N-lower alkyl substituted with one or more halogen atoms, OH, O-lower alkyl, O-lower alkyl substituted with one or more halogen atoms, CO-lower alkyl, CO-lower alkyl substituted with one or more halogen atoms, COOH, lower alkyl-COOH, CONH₂, CON-lower alkyl, SO₃H, SO₂NH₂, or SO₂N-lower alkyl; and

20 Z is a bond, O, S, lower alkyl radical, or lower heteroalkyl radical; or a pharmaceutically-acceptable salt thereof; and

25 an effective amount of a β -lactam antibiotic.

2. The method of Claim 1 wherein the β -lactam antibiotic is amoxicillin or ceftazidime.

3. A method of treating a bacterial infection comprising administering to an animal suffering from such an infection an effective amount of a compound having the formula:



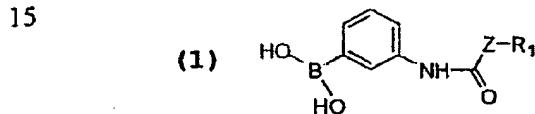
wherein:

R_1 is lower alkyl, lower alkyl substituted with one or more halogen atoms, a cyclic alkene, or a heterocyclic alkene, wherein the cyclic alkene or heterocyclic alkene may be substituted with one or more substituents R_2 ;

5 each R_2 is independently H, a halogen atom, lower alkyl, lower alkyl substituted with one or more halogen atoms, NH, NO, NO₂, CN, N-lower alkyl, N-lower alkyl substituted with one or more halogen atoms, OH, O-lower alkyl, O-lower alkyl substituted with one or more halogen atoms, CO-lower alkyl, CO-lower alkyl substituted with one or more halogen atoms, COOH, lower alkyl-COOH, CONH₂, CON-lower alkyl, SO₃H, SO₂NH₂, or SO₂N-lower alkyl; and

10 Z is a bond, O, S, lower alkyl radical, or lower heteroalkyl radical; or a pharmaceutically acceptable salt thereof.

4. A method of inhibiting a β -lactamase comprising contacting the β -lactamase with an effective amount of a compound having the formula.



wherein:

20 R_1 is lower alkyl, lower alkyl substituted with one or more halogen atoms, a cyclic alkene, or a heterocyclic alkene, wherein the cyclic alkene or heterocyclic alkene may be substituted with one or more substituents R_2 ;

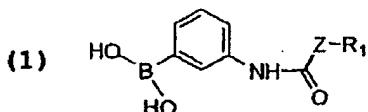
25 each R_2 is independently H, a halogen atom, lower alkyl, lower alkyl substituted with one or more halogen atoms, NH, NO, NO₂, CN, N-lower alkyl, N-lower alkyl substituted with one or more halogen atoms, OH, O-lower alkyl, O-lower alkyl substituted with one or more halogen atoms, CO-lower alkyl, CO-lower alkyl substituted with one or more halogen atoms, COOH, lower alkyl-COOH, CONH₂, CON-lower alkyl, SO₃H, SO₂NH₂, or SO₂N-lower alkyl; and

30 Z is a bond, O, S, lower alkyl radical, or lower heteroalkyl radical; or pharmaceutically acceptable salts thereof.

5. The method of Claim 4 wherein the β -lactamase is produced by bacteria, and the bacteria are contacted with the compound or salt thereof.

6. The method of Claim 4 wherein the contacting takes place *in vitro*.

7. A pharmaceutical composition comprising a pharmaceutically-acceptable carrier and a compound having the formula:



5

wherein:

R₁ is lower alkyl, lower alkyl substituted with one or more halogen atoms, a cyclic alkene, or a heterocyclic alkene, wherein the cyclic alkene or heterocyclic alkene may be substituted with one or more substituents R₂;

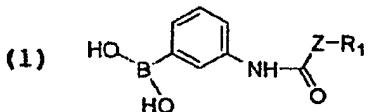
10 each R₂ is independently H, a halogen atom, lower alkyl, lower alkyl substituted with one or more halogen atoms, NH, NO, NO₂, CN, N-lower alkyl, N-lower alkyl substituted with one or more halogen atoms, OH, O-lower alkyl, O-lower alkyl substituted with one or more halogen atoms, CO-lower alkyl, CO-lower alkyl substituted with one or more halogen atoms, COOH, lower alkyl-COOH, CONH₂, CON-lower alkyl, SO₃H, 15 SO₂NH₂, or SO₂N-lower alkyl; and

Z is a bond, O, S, lower alkyl radical, or lower heteroalkyl radical; or pharmaceutically-acceptable salts thereof.

8. The composition of Claim 7 further comprising a β -lactam antibiotic.

9. The composition of Claim 8 wherein the β -lactam antibiotic is amoxicillin 20 or ceftazidime.

10. A compound having the formula:



25

wherein:

R₁ is lower alkyl, lower alkyl substituted with one or more halogen atoms, a cyclic alkene, or a heterocyclic alkene, wherein the cyclic alkene or heterocyclic alkene may be substituted with one or more substituents R₂;

30 each R₂ is independently H, a halogen atom, lower alkyl, lower alkyl substituted with one or more halogen atoms, NH, NO, NO₂, CN, N-lower alkyl, N-lower alkyl substituted with one or more halogen atoms, OH, O-lower alkyl, O-lower alkyl substituted

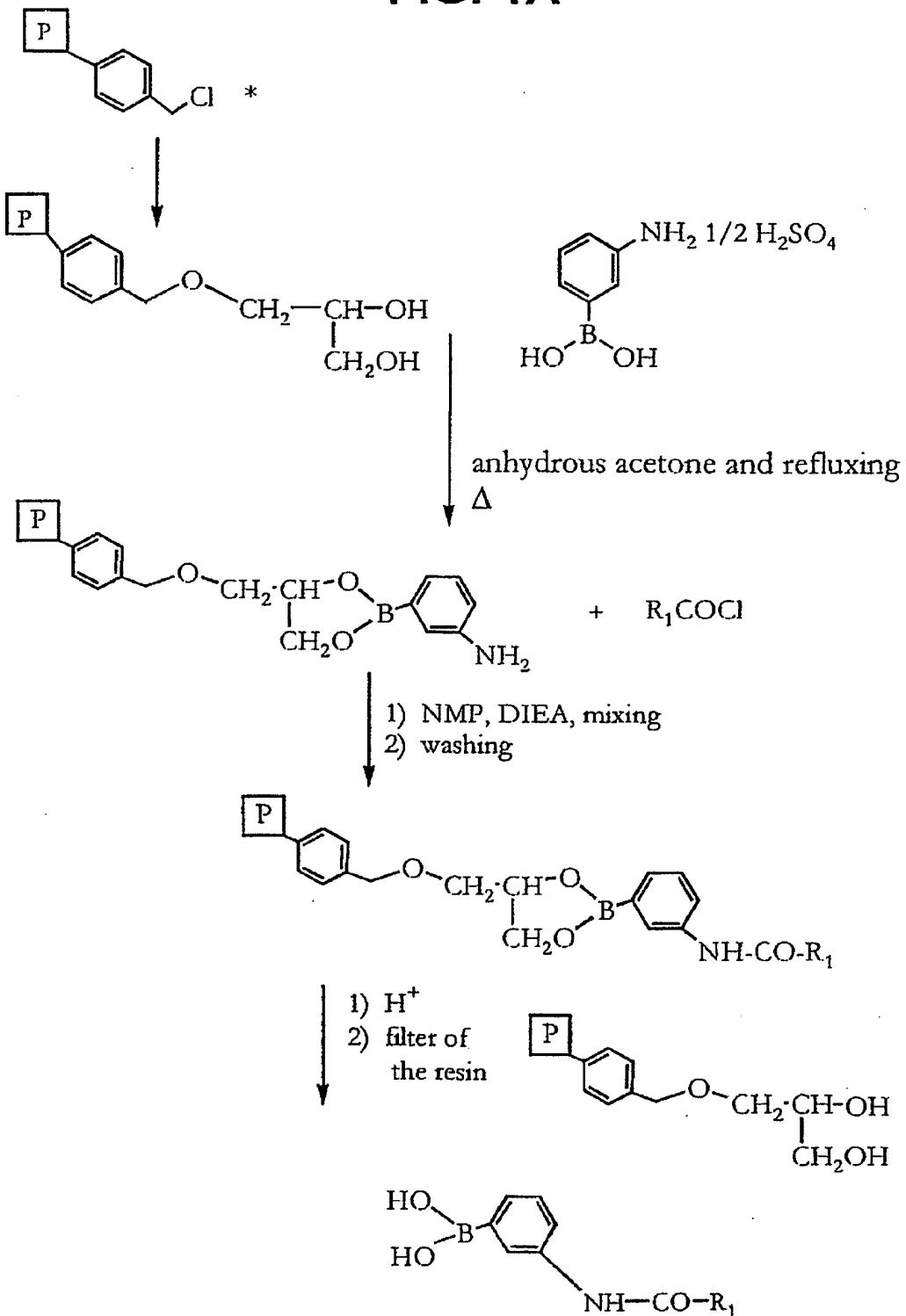
with one or more halogen atoms, CO-lower alkyl, CO-lower alkyl substituted with one or more halogen atoms, COOH, lower alkyl-COOH, CONH₂, CON-lower alkyl, SO₃H, SO₂NH₂, or SO₂N-lower alkyl; and

Z is a bond, O, S, lower alkyl radical, or lower heteroalkyl radical;

5 or pharmaceutically-acceptable salts thereof.

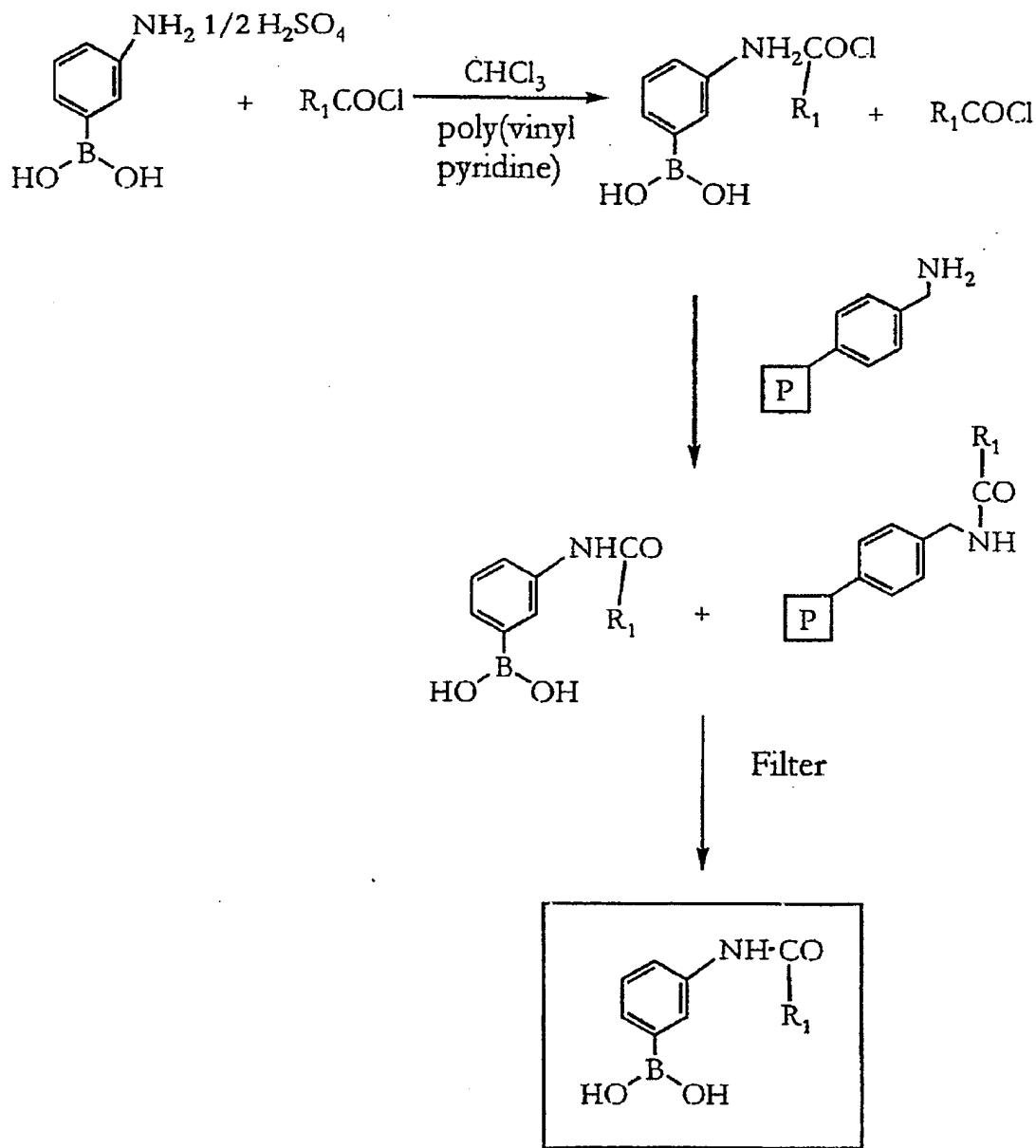
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FIG. 1A



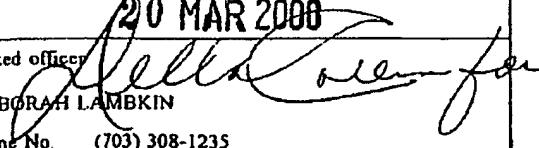
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FIG. 1B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/29956

A. CLASSIFICATION OF SUBJECT MATTER IPC(7) :C07D 333/56; C07C 233/00; A61K 31/38, 31/165 US CL :549/57; 564/176; 514/443, 617 According to International Patent Classification (IPC) or to both national classification and IPC										
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 549/57; 564/176; 514/443, 617										
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched										
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)										
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left; padding: 2px;">Category*</th> <th style="text-align: left; padding: 2px;">Citation of document, with indication, where appropriate, of the relevant passages</th> <th style="text-align: left; padding: 2px;">Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td style="padding: 2px;">A</td> <td style="padding: 2px;">US 5,482,974 A (PHILLION ET AL) 09 January 1996 (9/1/96), see abstract.</td> <td style="padding: 2px;">1-10</td> </tr> <tr> <td style="padding: 2px;">A,E</td> <td style="padding: 2px;">US 6,020,531 A (SHIRAIWA ET AL) 01 February 2000 (1/2/2000), see abstract.</td> <td style="padding: 2px;">1-10</td> </tr> </tbody> </table>		Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	A	US 5,482,974 A (PHILLION ET AL) 09 January 1996 (9/1/96), see abstract.	1-10	A,E	US 6,020,531 A (SHIRAIWA ET AL) 01 February 2000 (1/2/2000), see abstract.	1-10
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<input type="checkbox"/> Further documents are listed in the continuation of Box C <input type="checkbox"/> See patent family annex.										
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